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14. ABSTRACT Huanglian is a botanical prepared from the root of Coptis chinensis and deltoidea. We reported that huanglian inhibits growth of breast cancer cells in vitro in a dose-dependent manner (Li X. et al. Molecular Pharmacology, 58: 1287-1293, 2000). Based on these results, we developed huanglian, as an herbal extract, packaged in 250 mg capsules suitable for human clinical trials. We have treated 26 patients on the phase I trial of huanglian. We observed grade 3 diarrhea in one of six patients at cohorts 6 (5.25 gm/day) and 8 (8.25 gm/day), respectively. However, because of capsule number at this highest dose (33 capsules/day in 4 divided doses), further dose escalation did not appear feasible. We therefore elected to increase the amount of huanglian in the capsules from 250 to 500 mg. It took over a year to identify a new source of root from China that would meet our strict "biochemical" profile for Huanglian. With 500 mg capsules we have now been able to escalate to a dose of 10 gm/day. We have had one patient with dose-limiting nausea and vomiting and the cohort is expanded to 6 patients. We anticipate recommending a dose of 8 gm/day for phase II trials.					
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Introduction

Huanglian is a botanical agent prepared as a tea from the roots of *Coptis chinensis*. In traditional Chinese medicine it has been used to treat inflammatory conditions ranging from gastroenteritis to acute febrile illnesses with no reported toxicity. We tested huanglian for activity against cancer at MSKCC. We reported that huanglian potently inhibits the growth of a number cancer cells in vitro in a dose-dependent manner, with maximal inhibition at low micromolar concentrations (Li X. et al. Molecular Pharmacology, 58:1287-1293, 2000). MCF-7 and MDA-468 breast cancer lines were particularly sensitive to huanglian. The activity of huanglian was greater than an equivalent concentration of its major component, berberine, suggesting that several components contribute to its anticancer effect. It was therefore decided to take whole huanglian to human trial, as a novel departure from the conventional approach in drug development in which a single active compound is selected and tested. In addition to single agent activity against breast cancer cell lines, huanglian was also shown to enhance the effect of paclitaxel, supporting the future development of huanglian in combination with paclitaxel for the treatment of patients with metastatic breast cancer.

Research Aims/Key Research Accomplishments

The overall goal for this grant is to develop new therapeutic approaches in the treatment of patients with metastatic breast cancer based utilizing the Chinese botanical huanglian. The specific aims are to:

- 1) To conduct a phase I clinical trial of huanglian with both toxicity and efficacy endpoints.
- 2) Based on the results of the phase I clinical trial of single agent huanglian, conduct a phase II clinical trial of huanglian either as a single agent or in combination with paclitaxel in the treatment of patients with metastatic breast cancer
- 3)

Reportable Outcomes

1. "A Phase I Study of the Chinese Herb Huanglian (*Coptis chinensis*) in Patients with Advanced Solid Tumors" (MSKCC Protocol Number 00-061A(6): The purpose of this phase I study is to determine the optimal dose of huanglian for future phase II trials. Patients with advanced solid tumors who have failed all conventional therapy or for which there is no conventional therapy are eligible for this study. Twenty-one patients have been registered to this study. One patient elected to withdraw consent after study registration and never received huanglian.

i) Study design, defining the MTD, and best response: The initial study design utilized a rapid dose escalation schedule of 1 patient/level and the huanglian dose was to be increased by 50% in successive cohorts. The starting dose of huanglian was 1 gm/day or one capsule (250 mg/tablet), p.o., 4x/day. At dose level 3 (2.25 gm/day), one additional patient was added since the first patient developed progression of disease (POD) before completing her assessment for toxicity. Using this study design, we safely escalated to a

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dose of 3.5 gm/day or 14 capsules in 4 divided doses. At a dose of 5.25 gm/day (21 capsules/day), one patient developed grade 3 diarrhea (DLT) and the cohort was expanded to 6 patients with no further DLTs noted. However, because of this toxicity, the study design now changed to a classic dose escalation schema of 3 to 6 patients/dose level and a 25% dose escalation in all successive cohorts. Utilizing this approach, we escalated to a dose of 6.56 gm/day (26 capsules/day) in 3 patients without DLT. In the next cohort of 8.25 gm/day (33 capsules/day), we again observed 1 patient with grade 3 diarrhea. This cohort was expanded to 6 patients and no other DLTs were observed.

At this point of the study, it became evident that further dose escalation was not feasible simply based on capsule number. Yet, an MTD dose level had yet to be reached. Therefore, we elected to repackage the huanglian as 500 mg (rather than 250 mg capsules). As we had depleted our 250 mg capsule supply, we decided to obtain additional coptis Chinesis from China so as to produce a new water soluble and heat extratable supply of huanglian for the clinical trial. This approach proved to be a daunting task. We first tested several sources of coptis Chinesis root from Chinatown in NY but none met the “biochemical” profile of huanglian that was required for our clinical trial. Ultimately, we found a source in China which met our biochemical profile as defined by the HPLC peaks, berberine content, and inhibition of tumor cell growth in an in vitro cell proliferation assay. Since the capsule dose was increased, we elected to reopen the study at a starting dose of 8.0 gm/day (cohort 7b, 500mg/tablet, p.o., 4xday). In this cohort one participant experienced grade 1 diarrhea and another patient experienced grade 1 and grade 2 diarrhea, neither of which were considered dose-limiting. Therefore, cohort 8 at a dose of 10.0 gm/day was initiated. At this dose level one patient with breast cancer experience grade 3 nausea and vomiting that was refractory to anti-emetics. In view of this, the cohort has been expanded. We have treated another patient at this dose level without DLT.

These results from this study are summarized in the table below, which also indicates stable disease as best response in several patients on the study. Each of these patients had been progressing under observation or on therapy before entering the clinical trial with huanglian.

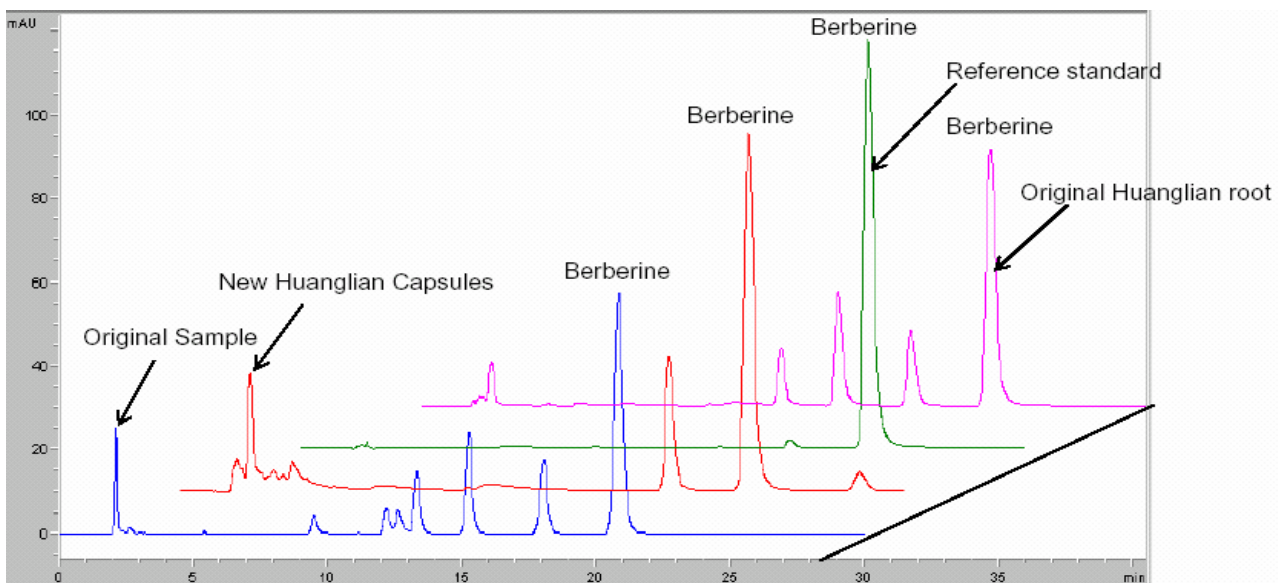
Cohort	Pt. #	Dose (gm/day)	Pill #	Toxicity	Best Response
1	1	1.0	4	0	Stable (colon): 6.4 months
2	1	1.5	6	0	Stable (neuro): 12 months
3	2	2.25	9	0 (1 NE)	None
4	1	3.5	14	0	None
5	6	5.25	21	1: gr. 3 diarrhea	Stable (sarcoma): 6.5 months
6	3	6.56	26	0	Stable (renal): 5.2 months
7	6	8.25	33	1: gr 3 diarrhea	Stable (sarcoma): 4.5 months
7b	3	8.0	16	3: 2 gr 1e diarrhea, 1 gr 2 diarrhea (no DLTs)	None

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8	2	10.0	20	1: gr 3 nausea/vomitting	None
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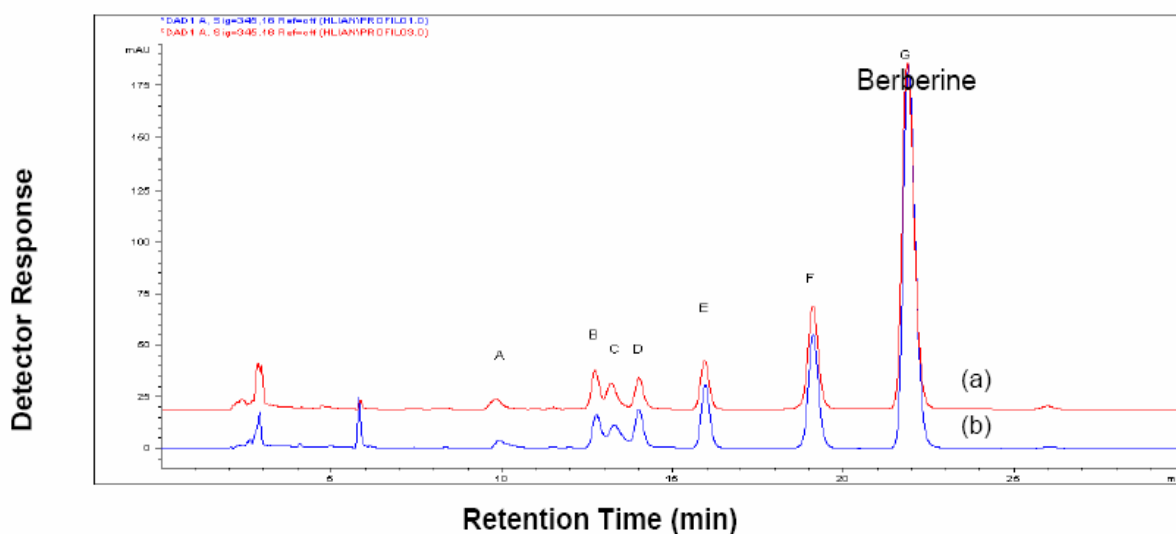
iii) Development of 500 mg huanglian capsules and producing sufficient quantity to complete the phase I clinical trial and to conduct the phase II clinical trial in advanced breast cancer:

Our plan has been to complete this phase I study of huanglian, so as to define the MTD and then test this in phase II clinical trials. We have been especially encouraged by stable disease in patients with advanced cancers (breast, renal, sarcoma, and neuroendocrine tumors) who were progressing either under observation or on chemotherapy. These patients had no other treatment options at the point of study entry. At the time of our last review we had identified a lot of raw Coptis Chinesis root that met our biochemical specifications for huanglian. However, 300 kg of the raw root was forwarded to Phoenix Laboratories for processing into 500 mg capsules of the heat extractable, water soluble fraction of huanglian for the clinical trial. Unfortunately, the processed material did not meet our specifications and, in fact, it contained 2 (not 7) peaks with only 11% (not 20 to 25%) berberine:

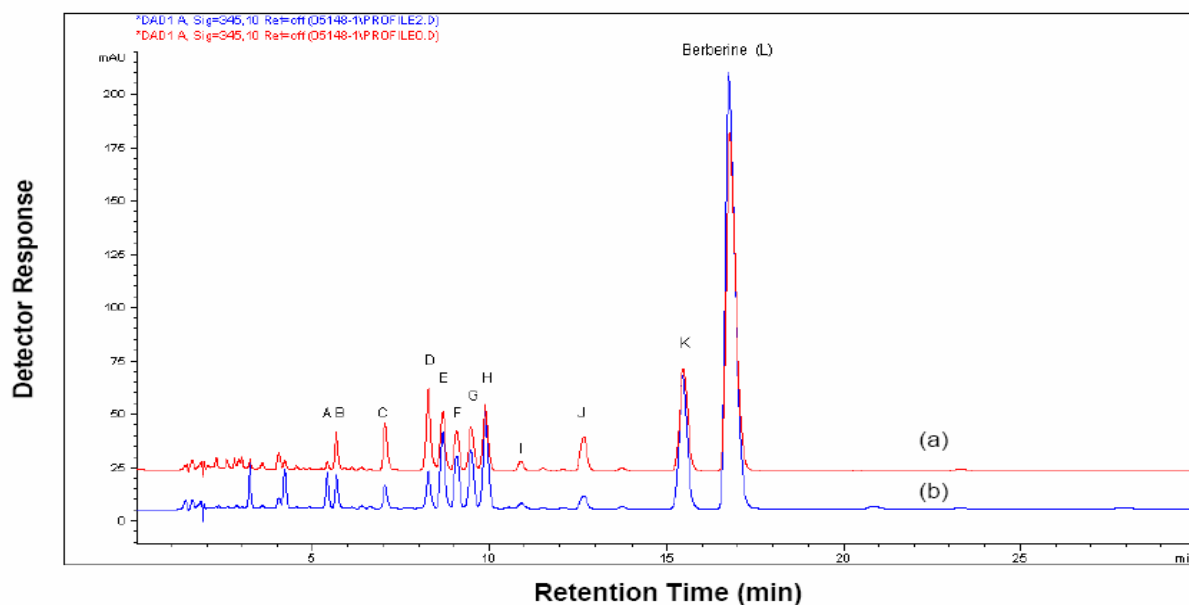


As shown above, the “new huanglian capsules”, obtained after extraction, shows only 2 HPLC peaks with one of the two being berberine. This contrasts with our original huanglian obtained at the start of the clinical trial and the original huanglian root obtained before the initiation of the current extraction process (each with 7 peaks by HPLC with the dominant peak being berberine). We were able to confirm these results.

In view of this we had to identify a new supplier as well as new contractor for 500 mg capsule production. The current lot of Coptis Chinesis (Lot 05148-1) was obtained from SiChuan Province in China.



As shown for the chromatographs the new lot (05158-1) has an HPLC profile that is essentially identical to the lot (68622) obtained in 2001 for our original clinical trials. Furthermore, we have updated our HPLC test procedures and have now identified additional peaks on the HPLC chromatographs.

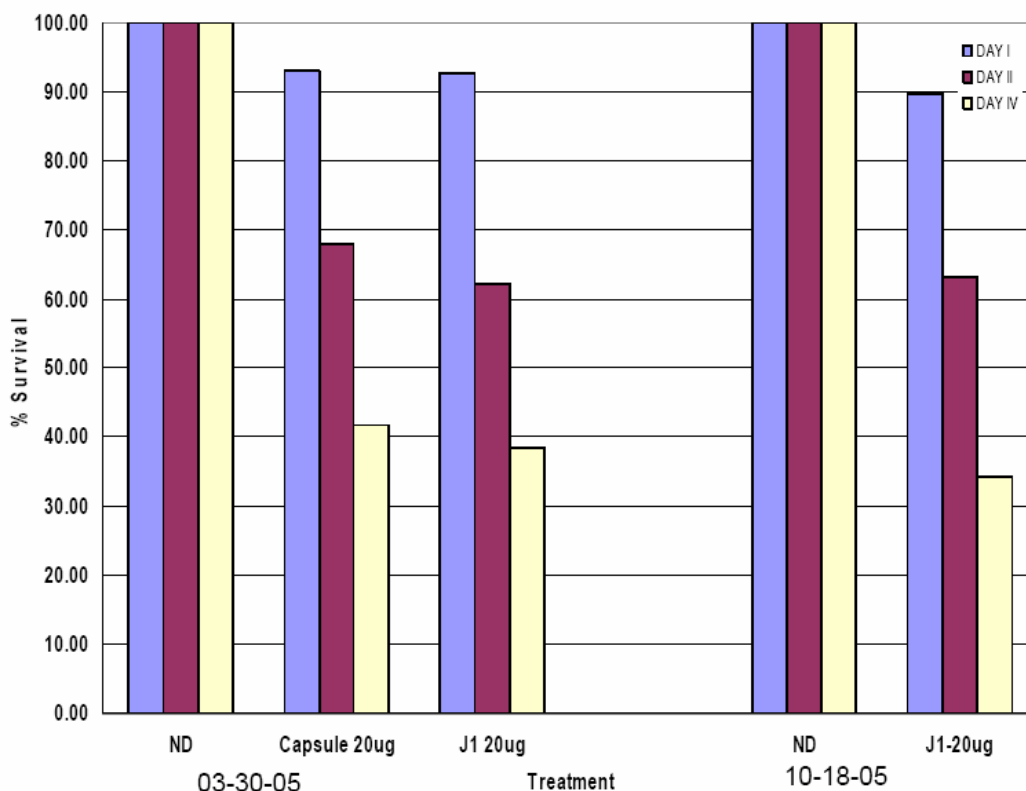


However, even using this new updated technique, the HPLC chromatograph of our current lot (b) is essentially identical to the original lot (a).

Before proceeding to clinical trial, our “biochemical profile” also required confirming biologic activity. We therefore evaluated biologic activity with this new lot. For these studies MKN-74 gastric cancer cells were exposed to the original huanglian obtained from clinical capsules or from huanglian obtained from root preparation and extraction (J1). The cells were exposed to 20 µg/ml of test material for 1, 2 and 4 days

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and growth inhibition was then determined in an established SRB assay. Results are shown below and are expressed as percent cell survival as compared to untreated controls.



As shown above, the results from 3-30-05 indicate that current lot (J1) inhibited cell growth to the same degree as the huanglian obtained from our original extraction and used in the capsules for the clinical trial. J1 was then used to make huanglian lot 05148-1. The biological activity of this lot was first tested on 10-18-05 and the results are shown in the figure above. As shown lot 05148-1 inhibits tumor cell growth in a manner that is identical to that of the original capsules.

Finally, our “biochemical profile” required establishing a huanglian berberine content of at least 20%. Our analysis confirmed this. Furthermore, our updated methods for HPLC analysis resulted in improved separation efficiency and robustness. Because of this, we have redefined our required berberine content for future huanglian extractions from 20 % to 30 to 17.0 to 27.0% (as free base).

Conclusions

This process of drug development does illustrate the complexities of bringing botanical medicine into the area of clinical cancer therapy. Ensuring a reliable source of material that can be reproducibly called “huanglian” is critical for the success of this program. Our efforts clearly have implications for other investigators attempting to develop botanical medicines for breast cancer therapy. In order to do this in a meaningful and

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reproducible manner, it will be necessary to establish and maintain strict criteria so as to produce an “acceptable” drug product that is suitable for clinical cancer use. However, as we have learned, this is not easy, and even small deviations can result in the production of material that fails to meet these standards. Nevertheless, despite these difficulties, we have succeeded in identifying and producing sufficient amounts of huanglian (100,000 capsules) which should allow us to complete the phase I clinical trial of single agent huanglian and then initiate a phase II clinical trial of huanglian.